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MOLECULAR AND BIOLOGIC CHARACTERISTICS OF *TOXOPLASMA GONDII* ISOLATES FROM WILDLIFE IN THE UNITED STATES

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ABSTRACT: *Toxoplasma gondii* isolates can be grouped into 3 genetic lineages. Type I isolates are considered more virulent in outbred mice and have been isolated predominantly from clinical cases of human toxoplasmosis, whereas types II and III isolates are considered less virulent for mice and are found in humans and food animals. Little is known of genotypes of *T. gondii* isolates from wild animals. In the present report, genotypes of isolates of *T. gondii* from wildlife in the United States are described. Sera from wildlife were tested for antibodies to *T. gondii* with the modified agglutination test, and tissues from animals with titers of 1:25 (seropositive) were bioassayed in mice. *Toxoplasma gondii* was isolated from the hearts of 21 of 34 seropositive white-tailed deer (*Odocoileus virginianus*) from Mississippi and from 7 of 29 raccoons (*Procyon lotor*); 5 of 6 bobcats (*Lynx rufus*); and the gray fox (*Urocyon cinereoargenteus*), red fox (*Vulpes vulpes*), and coyote (*Canis latrans*) from Georgia. *Toxoplasma gondii* was also isolated from 7 of 10 seropositive black bears (*Ursus americanus*) from Pennsylvania by bioassay in cats. All 3 genotypes of *T. gondii* based on the SAG2 locus were circulating among wildlife.

Toxoplasma gondii infections are widely prevalent in human beings and animals worldwide (Dubey and Beattie, 1988; Tenter et al., 2000). Postnatally, humans become infected by ingesting tissue cysts from undercooked meat or consuming food or drink contaminated with oocysts. However, only a small percentage of exposed adult humans develop clinical signs after exposure. It is not known whether the severity of toxoplasmosis in immunocompetent persons is due to the parasite strain, host variability, or other factors.

Overall, there is low genetic diversity among the *T. gondii* isolates examined so far. *Toxoplasma gondii* isolates have been classified into 3 genetic types (I, II, III) based on restriction fragment length polymorphism (RFLP) (Howe and Sibley, 1995; Howe et al., 1997; Grigg, Bonnefoy et al., 2001). It was suggested that isolates of type I and II are more likely to result in clinical toxoplasmosis in humans, but genetic characterization has been limited essentially to isolates from patients ill with clinical toxoplasmosis (Howe et al., 1997; Fuentes et al., 2001; Grigg, Ganatra et al., 2001; Aspinall et al., 2003). However, most isolates of *T. gondii* from domestic animals from the United States and Europe are type II or type III (Howe and Sibley, 1995; Mondragon et al., 1998; Owen and Trees, 1999; Jungersen et al., 2002). In a recent study, 17 of the 25 isolates of *T. gondii* obtained from asymptomatic free-range chickens from the rural areas surrounding São Paulo, Brazil, were type I (Dubey, Graham et al., 2002).

Most of the research in *T. gondii* has been focused on humans or domestic animals. The increasing urbanization of the U.S. landscape has resulted in greater interaction between humans and wild fauna, including raccoons, coyotes, and white-tailed deer, that survive well in close contact with humans. Wildlife species that live in urban areas are increasingly likely to come into contact with both domestic cats and the large population of feral cats that exist in some cities. In other areas, large mammals such as white-tailed deer and black bears are popular game animals for both sport and meat hunting. Little is known about the prevalence and distribution of genotypes of *T. gondii* in these wildlife species. In this study, we describe the genetic and biologic characteristics of isolates of *T. gondii* from several species of wildlife in the United States.

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MATERIALS AND METHODS

Animals surveyed

Raccoons (*Procyon lotor*), bobcats (*Lynx rufus*), coyotes (*Canis latrans*), gray fox (*Urocyon cinereoargenteus*), and red fox (*Vulpes vulpes*) were trapped in either the Atlanta area (DeKalb County) or the Albany area (Baker County) of Georgia as part of an epidemiologic study on *Dracunculus insignis* (Table I). Hearts were collected from animals, placed in zip-lock bags, and transported overnight to the Animal Parasitic Diseases Laboratory at Beltsville, Maryland.

Samples from adult (1–7 yr) white-tailed deer (*Odocoileus virginianus*) were obtained from collections in Mississippi during 2002 and 2003. They were obtained in 5 groups from 6 sites spanning a broad geographic area of the state. Group 1 deer were from Camp McCain military reservation in Granada County and were killed on 15 February 2002. Group 2 deer were from Calhoun County Wildlife Management area, Calhoun County, and Woodlawn hunting club, Wilkinson County, and were killed on 12 March 2002. Group 3 deer were from Little Biloxi Wildlife Management Area, Stone County, and were killed on 3 April 2002. Group 4 deer were from Wolf River Wildlife Management Area, Lamar County, and were killed on 4 April 2002. Group 5 deer were from Duck Lake hunting club, Tunica County, and were killed on 10 March 2003. Between the time of death and the bioassay in mice in the 5 groups of deer, 12, 8, 8, 7, and 8 days elapsed, respectively. During this time, hearts were kept cold (4–10 C).

Samples from black bears (*Ursus americanus*) were obtained from those hunted in Pennsylvania. The Pennsylvania Game Commission requires that all hunter-killed black bears be examined and tagged at check stations located throughout the state. During the hunting season of 1998, hunters were requested to save the hearts from bears killed on 22 November 1998. Hearts were transported to the Indiana University of Pennsylvania, where blood was collected from the heart and centrifuged, and the serum was separated. Hearts and sera were kept cool (refrigerated or on ice) and transported to Beltsville. In addition, *T. gondii* isolates obtained previously from the hearts of bears from Pennsylvania killed in 1993 (Dubey, Humphreys, and Thulliez, 1995) were genotyped.

TABLE I. Genetic lineages of *Toxoplasma gondii* isolates from wildlife animals from Georgia and Mississippi.

			T. gondii isolates			
				Type		
Host	No.	% Seropositive	No.	I	II	III
Raccoons	75	52	7	2 (4*, 5)†	2 (4, 1)†	3 (5, 5, 1)†
Bobcats	6	83	5	0	5 (5 in 2, 4 in 2, 3 in 1)†‡	0
Gray fox	2	50	1	0	1 (5)†	0
Red fox	1	100	1	0	1 (5)†	0
Coyote	1	100	1	0	1 (5)†	0
Deer	73	46	21	0	21 (5 in 15, 4 in 2, 3 in 1, 2 in 2, 1 in l)†§	0

* All 4 mice died 8–20 days PI.
† No. of mice infected with *T. gondii* of 5 mice inoculated.
‡ Three of 5 infected mice, inoculated with heart tissue from 1 bobcat, died 8, 18, and 31 days PI.
§ All mice inoculated with deer tissues survived except in 4 instances where 1, 1, 1, and 2 infected mice died between 18 and 24 days PI.

Serologic examination for *T. gondii*

Serum samples, often obtained from heart, were tested for antibodies to *T. gondii* with the modified agglutination test (MAT) as described (Dubey and Desmonts, 1987). Sera were diluted 2-fold starting at the 1:25 dilution.

Bioassay for *T. gondii* in mice

Brains or hearts, or both, were bioassayed in outbred female Swiss Webster mice obtained from Taconic Farms, Germantown, New York, as reported previously (Dubey, Graham et al., 2002). Briefly, the heart (50 g or the whole heart) of each animal was homogenized, digested in acidic pepsin and washed, and the homogenate was inoculated subcutaneously into 5 mice (Dubey, 1998). Tissue imprints of mice that died were examined for *T. gondii* tachyzoites or tissue cysts. Survivors were bled 5 wk postinoculation (PI), and a 1:25 dilution of serum from each mouse was tested for *T. gondii* antibodies with the MAT. Mice were killed 62 days PI, and their brains were examined for tissue cysts as described (Dubey and Beattie, 1988).

Bioassay of bear tissues for *T. gondii* in cats

Cats were used for bioassays because larger volumes of meat can be fed to them than can be inoculated into mice and because diagnosis can be made quickly in live cats by examination of their feces for oocysts. The cats used were 3 to 12 mo old and were from a *T. gondii*–free cat colony described previously (Dubey, 1995). The cats had no detectable *T. gondii* antibody in the MAT in 1:25 dilution of their serum. Bear sera were screened at dilutions of 1:25, 1:50, and 1:500 for *T. gondii* antibodies in the MAT on the day of arrival in Beltsville. Hearts from 10 bears were selected for bioassay, depending on the antibody titer and the degree of autolysis of heart tissue. For feeding to cats, 95–500 g of heart tissue was ground in a blender and fed to a cat over a period of 2–4 days. In the interim, the heart tissue was stored at 4 C. Feces from cats were collected daily from the first day they were fed hearts until 21 days after the ingestion of the last cardiac meal. Feces were examined for *T. gondii* oocysts using the sucrose solution procedure. Oocysts were collected from the flotation preparations, sedimented in water, and aerated in 2% H_2SO_4 at room temperature for at least 7 days (Dubey and Beattie, 1988). Sporulated oocysts were neutralized with 3.3% aqueous NaOH and inoculated orally into 8 mice. The inoculated mice were examined for *T. gondii* tachyzoites or tissue cysts. Tissues containing tachyzoites or tissue cysts were subinoculated into 2–5 mice to confirm that they were *T. gondii*.

Genotyping of *T. gondii* isolates

Samples of lungs from the mice that died and the brains of mice that survived for 2 mo after inoculation with tissues of animals, except bear tissue, were frozen at −70 C for DNA characterization as described (Lehmann et al., 2000). Different procedures were used to isolate DNA from the isolates from black bears. Oocysts of each isolate were heated to 65 C for 10 min to kill *T. gondii* and then processed for DNA. Alternatively, tachyzoites from the mesenteric lymph nodes of mice that died or were killed 1 wk PI, or from the brains of mice that survived for 1 mo or more, after they were fed oocysts were used for DNA extraction. About 5 g of each bear heart was also used for the extraction of DNA. Polymerase chain reaction–RFLP genotypes of the SAG2 locus were used to genetically characterize the isolates (Howe et al., 1997).

RESULTS

Antibodies to *T. gondii* were found in 39 of 75 (52%) raccoons. Titer levels varied substantially between individuals, with titers of 1:25 in 6, 1:50 in 15, 1:100 in 4, and 1:200 in 14. *Toxoplasma gondii* was isolated from 7 of 33 seropositive (1:25 or more) raccoons bioassayed (Table I). All parasitologically proven raccoons were from DeKalb county, and their titers were 1:50 in 1, 1:100 in 1, 1:200 in 3, and 1:400 in 2.

Five of the 6 bobcats sampled had *T. gondii* antibodies with titers of 1:100 in 1 and 1:200 in 4. Viable *T. gondii* was isolated from all seropositive bobcats (Table I). All parasitologically proven bobcats were from Albany County.

Antibodies to *T. gondii* were found in 1 (titer 1:200) of the 2 gray foxes, in the red fox (titer 1:50), and in the coyote (titer 1:200) (Table I). *Toxoplasma gondii* was isolated from the samples from seropositive foxes and the coyote; all were from Albany County (Table I).

Toxoplasma gondii antibodies were found in 34 of 73 (46.5%) white-tailed deer with titers of 1:25 in 5, 1:50 in 4, 1:100 in 9, and 1:200 in 16. Seroprevalences varied among collection areas, with 33.3% (n = 33, group 1), 57.1% (n = 21, group 2), 50% (n = 6, group 3), 33.3% (n = 9, group 4), and 33.3% (n =12, group 5) being positive. Viable *T. gondii* was isolated from the hearts of 21 of 34 (61.7%) seropositive deer. The parasite isolation was 1 of 5 with titer of 1:25, 3 of 4 with titers of 1:50, 7 of 9 with titers of 1:100, and 10 of 16 deer with titers of 1:200.

Antibodies to *T. gondii* were found in 66 of 80 (82.5%) black bears killed in 1998, with titers of 1:25 in 6, 1:50 in 40, and 1:500 in 20. Cats fed hearts from 7 of 10 bears shed *T. gondii* oocysts (Table II). All mice fed oocysts from bear isolates died or became ill 4–7 days PI, and tachyzoites were found in their mesenteric lymph nodes. Mice inoculated with tachyzoites of the 6 isolates remained asymptomatic, whereas those inoculated with *T. gondii* isolate from bear no. 22 died of acute toxoplas-

TABLE II. Isolation of *Toxoplasma gondii* from the hearts of black bears killed in 1998 by bioassay in cats.

Bear no.	Antibody titer (MAT)	No. of the cat that shed oocysts	Genotype
2	500	397	III
5	500	386	II
7	50	385	II
10	500	379	II
12	500	396	III
15	500	389	II
22	500	392	I

mosis 14 days PI. Genotypes of the 7 isolates characterized were type I in 1, type II in 4, and type III in 2.

Toxoplasma gondii was isolated from the hearts of 10 of the 28 bears killed in 1993. Seven of the isolates were obtained by bioassay in cats, 2 isolates were obtained by bioassay in mice, and 1 isolate was obtained by bioassay in both cat and mice (Dubey, Humphreys, and Thulliez, 1995). Howe and Sibley (1995) used oocysts of 7 of these isolates for genotyping. They identified 4 of these as type III, 2 as type II, and 1 as a recombinant of types II and III. The bear isolate designations used by Howe and Sibley (1995) refer to cats nos. B75, B74, B5, B73, B41, B62, and B70 and correspond to bears nos. 1, 2, 4, 6, 8, 9, and 10 of Dubey, Humphreys, and Thulliez (1995). The genotypes of the 2 isolates obtained by bioassays in mice were type III in 1 and type II in 1 (present study). Thus, of the 16 *T. gondii* isolates from Pennsylvania bears studied, type III lineage was found in 7, type II in 7, type I in 1, and recombinant of types II and III in 1.

All 3 genotypes of *T. gondii* could be isolated from raccoons and bears, whereas the other 29 isolates (from deer, bobcats, foxes, and coyote) were of type II (Table I).

DISCUSSION

Among all hosts of *T. gondii* in the United States, and perhaps in the world, the prevalence of *T. gondii* is the highest among black bears in the eastern United States. Antibodies to *T. gondii* have been found in approximately 80% of black bears, and the parasite has been isolated in 70% of seropositive animals. Using a cutoff titer of 1:25 in the MAT, antibodies were found in 80% of 665 (Briscoe et al., 1993) and 79% of 28 (Dubey, Humphreys, and Thulliez, 1995) bears from Pennsylvania and 84% of 143 bears from North Carolina (Nutter et al., 1998). Remarkably, a similar seroprevalence (82%) was found in bears surveyed in the present study. Antibodies to *T. gondii* were found in 62 of 143 (43%) black bears from Alaska (Zarnke et al., 2000).

Toxoplasma gondii was isolated from 10 of 22 (45.4%) seropositive bears killed in 1993 (Dubey, Humphreys, and Thulliez, 1995) and 7 of 10 (70%) seropositive bears in the present study. The isolation data from the 1993 and 1998 studies are not comparable because in the 1993 study, all bear tissues were bioassayed irrespective of antibody status, whereas only selected bear hearts were bioassayed in the 1998 study.

Antibodies to *T. gondii* in white-tailed deer were also widely prevalent in the United States. Using a titer of 1:25 in MAT, antibodies to *T. gondii* were found in 44% of 106 deer from Kansas (Brillhart et al., 1994), 30% of 1,367 deer in Minnesota (Vanek et al., 1996), 44% of 16 deer from Alabama (Lindsay et al., 1991), 60% of 593 deer from Pennsylvania (Humphreys et al., 1995), and 46.5% of deer from Mississippi (the present study). Lindsay et al. (1991) isolated *T. gondii* from 4 of 19 deer: the isolates were from 4 of 6 seropositive (MAT titer of >1:50 or more) and none of 10 seronegative deer, and sera were not available from the 3 deer bioassayed. The isolation prevalence (66%) from seropositive deer from the study from Alabama (Lindsay et al., 1991) was comparable with the present study of 21 of 34 deer (61.7%). Deer are popular game animals in the United States, with between 250,000 and 350,000 killed each year in Mississippi alone (Mississippi Department of Wildlife, Fisheries, and Parks Deer Program Report 2000). Cases of clinical toxoplasmosis (Sacks et al., 1983), including ocular manifestations (Ross et al., 2001), have been documented in humans who had consumed undercooked venison.

Because of the high seroprevalence of *T. gondii* in bears and deer, hunters should be encouraged to wear gloves while skinning and handling game meat, wash their hands thoroughly afterward, either thoroughly cook meat or freeze it before consumption, and not leave discarded viscera exposed to carnivores, especially cats.

Seroprevalence of *T. gondii* in raccoons in the United States is also high (for review see Dubey and Odening, 2001). Using a cutoff value of 1:25 in MAT, antibodies were found in 48–70% of raccoons from the United States (Dubey et al., 1992; Brillhart et al., 1994; Dubey, Weigel et al., 1995; Mitchell et al., 1999). In the present study, *T. gondii* antibodies were found in 39 of 75 (52%) raccoons from Georgia, and *T. gondii* was isolated from 7 of 33 (21.2%) seropositive raccoons. In 1964, Walton and Walls (1964) found dye test antibodies in 31 of 67 (42%) raccoons from Fort Stewart, Georgia.

Antibodies to *T. gondii* were present in 5 of 6 bobcats, and viable parasites were recovered from all 5 seropositive bobcats. The only previous report of isolation of *T. gondii* from bobcats was that of Walton and Walls (1964); they recovered the parasite from the brain of 1 of 16 bobcats from Fort Stewart, Georgia, and 73% of 15 bobcats tested had antibodies to *T. gondii*. The higher isolation of viable *T. gondii* from raccoons and bobcats in the present study, compared with the study of Walton and Walls (1964), may be due to the technique used and the tissue bioassayed. The latter investigators used brain tissue for bioassay, and they inoculated homogenized brain suspension into mice, whereas in the present study, myocardium was used, and the tissues were digested in pepsin to release bradyzoites from intracellular tissue cysts before the bioassay in mice. With respect to sheep, goats, cats, and chickens, *T. gondii* may be more frequent in the muscle than in the brain (Dubey and Beattie, 1988). The high prevalence of *T. gondii* in bobcats is also important epidemiologically because bobcats can excrete *T. gondii* oocysts (Miller et al., 1972), and seroprevalence in bobcats in North America is high (Dubey and Odening, 2001; Labelle et al., 2001).

Most isolates of *T. gondii* from wildlife were relatively avirulent for mice (Tables I, II). All infected mice inoculated with 17 *T. gondii* infected deer hearts survived for 2 mo. Five of 20 mice infected with the remaining 4 isolates died of toxoplasmosis. All 4 mice infected with the *T. gondii* isolate from rac-

coon no. 008 died, and this isolate was determined to be type I. Three of 5 infected mice inoculated with isolate from 1 bobcat died of toxoplasmosis between 18 and 31 days PI, and this isolate was type II. Of interest is the survival (at least for 38 days) of all 5 mice infected with isolate from raccoon no. 029 because this strain was type I. The genotyping was repeated from this isolate because type I strains are considered to be fatal for mice, and the results were confirmed. Although 5 mice were inoculated with each tissue, unfortunately DNA was extracted from only 1 of these 5 mice for genotyping. The mortality of the bear isolates was not studied in a similar manner because primary isolations were made by feeding bear tissues to cats.

In the present study, assessment of virulence in mice and DNA isolation were performed using primary isolation, thus minimizing chances of enhanced virulence by subpassages in cell culture or mice. Howe and Sibley (1995) obtained 106 *T. gondii* isolates from many laboratories in the world and grouped them into 3 types (I, II, III). Among these 106 isolates were 4 recombinants (1 as types I and III and 3 as types II and III). Howe et al. (1997) subsequently reported that most *T. gondii* isolates from 68 clinical cases of human toxoplasmosis were type II (81%), with only a few being type I (10%) and type III (9%). Most *T. gondii* isolates in these investigations had been maintained for various periods of time in cell culture or mice, and thus selection might have affected the final genotyping data of *T. gondii*.

Recently, Fuentes et al. (2001), and Aspinall et al. (2003) used direct analysis of *T. gondii* DNA from clinical specimens from humans suffering from toxoplasmosis and reported surprising results. Direct amplifications were possible in 26 of 34 clinical samples (Fuentes et al., 2001). Of the 25 cases of toxoplasmosis from Spain, 10 were due to type I (40%), 10 to type II, and 5 to type III (Fuentes et al., 2001). Even more astonishing results were obtained by Aspinall et al. (2003), where of 32 toxoplasmosis patients from England and Wales, 10 had type I, 11 had type II, 10 had 2 types (I and II), and 1 had type III.

In the present study, direct amplification of *T. gondii* DNA was not successful using 5-g tissue samples from the 7 bear hearts infected with *T. gondii*. This is probably because the density of *T. gondii* in tissues of asymptomatic wild and domestic animals is too low to be detected in DNA extractions from ~250 μg of tissue homogenates. It is estimated that there may be only a few *T. gondii* in 100 g of meat, and these organisms may not be detectable even by bioassays in mice inoculated with tissue homogenates. For example, during the 1993 hunting season, *T. gondii* was isolated from 10 of 22 seropositive bears. Pepsin digests of 100 g of digested heart tissue were inoculated into 5 mice. Additionally, up to 650 g of undigested heart from 11 bears was fed to cats, and their feces were examined for shedding of *T. gondii* oocysts. *Toxoplasma gondii* was recovered from only 3 bears by bioassay in mice, and only 5 of 15 mice inoculated were infected, indicating that there were very few *T. gondii* in the inoculum (Dubey, Humphreys, and Thulliez, 1995). Cats fed with hearts from 7 bears shed *T. gondii* oocysts even though these hearts were negative by bioassay in mice.

It is of interest that in the present study, type I isolates were obtained from asymptomatic bears and raccoons. Type I isolates appear to be rare among animals in the United States, although only isolates from pigs have been examined in detail to support this statement. Among the 106 *T. gondii* isolates that Howe and Sibley (1995) analyzed, there were 2 type I strains (GT-1 from goat, CT-1 from beef cattle) and a recombinant of types I and III (P 89 from a pig) from food animals. All 3 strains were isolated by 1 of us (J.P.D.) and were selected for inclusion in the study of Howe and Sibley (1995) because of high virulence to mice. Both the white-tailed deer isolates that Howe and Sibley (1995) analyzed were type II, and these were isolated from deer in Alabama (Lindsay et al., 1991). All isolates from white-tailed deer in the present study were type II. Thus, only type II lineage has been isolated from deer in the United States. All 13 isolates of *T. gondii* from sea otters were also type II (Cole et al., 2000). Whether or not this is a coincidence needs investigation.

As stated earlier, humans become infected with *T. gondii* by ingesting tissue cysts or by ingesting beverages or food contaminated with oocysts excreted in the feces of an infected cat. Nothing is known of the genotypes of *T. gondii* isolates derived from the feces of naturally infected cats, and this information is vital because cats are essential for maintaining the circulation of *T. gondii* in nature.

Among food animals, pigs are the most likely source of *T. gondii* infection for humans in the United States because the prevalence of *T. gondii* in cattle and indoor-raised poultry is rare and little mutton is eaten on a national basis (Dubey, 1994). More information is available on genotypes of *T. gondii* isolates from pigs than from any other animal species. In 1 study, 170 viable isolates of *T. gondii* were obtained from the hearts of 1,000 sows (from several farms in Iowa) by bioassay in mice and cats (Dubey, Thulliez, and Powell, 1995). Of these 170 isolates, 108 were obtained by bioassay in mice, and an additional 62 were obtained by bioassay in cats. All mice inoculated with swine tissues survived for 2 mo, suggesting that *T. gondii* strains from these sows were of low virulence and not type I. Of these 170 isolates, 43 were selected for genotyping using the methods described by Howe and Sibley et al. (1997). Thirty-six of these 43 isolates were type II, and 7 were type III (Mondragon et al., 1998). One of these 170 isolates (P 89) was identified as a recombinant of types I and III strains (Howe and Sibley, 1995). Recently, *T. gondii* was isolated from 51 of 55 market-age pigs from a single farm (Dubey, Gamble et al., 2002). Based on mouse virulence data, none of these 51 isolates was likely to be type I because all the isolates were avirulent for mice (Dubey, Gamble et al., 2002). Of the 25 arbitrarily selected *T. gondii* isolates genotyped from these 51 isolates, 20 were type III, and 5 were type II (Lehmann et al., 2003).

It is interesting that there was no recombinant among the isolates that were obtained by feeding pig tissues to cats because there should have been opportunities for mating in the gut of the cat. Recombinants have been produced in experimentally infected cats (Su et al., 2002). Additionally, oocysts of these 25 isolates were fed to mice, and DNA was extracted from the mesenteric lymph nodes of mice killed 4–7 days later, minimizing chances for selection against multiple-genotype infection. However, this preliminary study was based on results with only 1 (SAG 2) locus. Studies are in progress at the laboratories of the Centers for Disease Control and Prevention (CDC) and the United States Department of Agriculture (USDA) in Atlanta and Beltsville to examine the genetic di-

versity of *T. gondii* using a large number of isolates obtained from domestic and wild animals.

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